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(57) Abstract

Oral care compositions comprising nisin, an antimicrobial agent and a dentally acceptable excipient or carrier are of use in the treatment or prophylaxis of plaque, periodontal disease and oral fungal infections.

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Mouthcare compositions.

This invention relates to oral hygiene compositions and in particular to mouthwashes and dentifrices having improved activity against a range of orally important pathogenic microorganisms, including bacteria and fungi.

Pathogenic oral bacteria are implicated in a number of conditions of the oral cavity, including plaque, gingivitis and periodontal disease. In addition, pathogenic fungi such as *Candida* may also be present in the oral cavity and give rise to disease states such as thrush requiring therapy.

Agents which in the past have been suggested for use as oral antibacterial agents include cationic species such as chlorhexidine, alexidine, hexetidine and cetyl pyridinium chloride as well as non-cationic species such as triclosan. Some of these antibacterial agents are also effective as antifungal agents.

More recently, it has been suggested that the polypeptide antibiotic nisin (Merck Index, 11th edn., entry 6481) may be of use in oral hygiene. Nisin is a lanthocin, comprising the atypical amino acid lanthionine, produced naturally by various strains of the bacterium *Streptococcus lactis*. It is also a naturally occurring preservative found in low concentration in milk and cheese. Nisin has recently been recognised by the FDA as a direct food ingredient. A summary of the properties of nisin is to be found in Advances in Applied Microbiology 27 (1981), 85-123. A purified form of nisin has recently been made available by Applied Microbiology Inc., under the trade name Ambicin N.

Oral care applications of nisin are disclosed in WO 93/11738 and WO 89/12399. The former discloses suitable dentifrice formulations whilst the latter discloses broad spectrum disinfectant compositions which may be in the form of oral rinses in which nisin is combined with a non-bactericidal agent such as a surfactant or a chelating agent, to extend the spectrum of activity of the composition.

In many instances, a single anti-microbial agent does not have a sufficiently broad spectrum of activity to deal adequately with a wide range of pathogenic microorganisms which may be found in the oral cavity. Combining different anti-microbial agents is not always successful as the presence of one may antagonise the activity of the other.

We have now surprisingly found that nisin may be effectively combined with an antimicrobial agent, without compromising the activity of nisin or the antimicrobial agent.

Accordingly, the present invention provides an oral hygiene composition which comprises an antibacterially effective amount of nisin, an antimicrobially effective amount of an antimicrobial agent and an orally acceptable carrier or

excipient.

Suitable antimicrobial agents may have antibacterial or antifungal activity or a combination of antibacterial and antifungal activity.

Suitable antimicrobial agents for use in compositions of the present invention include cationic antimicrobial agents and non-cationic antimicrobial agents, as well as other polypeptide antibiotics (bacteriocins) and antifungal agents. Accordingly, in a preferred embodiment, the present invention provides an oral hygiene composition comprising an antibacterially effective amount of nisin, an antimicrobially effective amount of an antimicrobial agent which is a cationic antimicrobial agent, a non-cationic antimicrobial agent, another (non-nisin) polypeptide antibiotic (bacteriocin) or an antifungal agent, and an orally acceptable carrier or excipient

Compositions of the present invention have a broader range of antimicrobial activity. For instance, nisin has limited activity against *Candida* species whereas agents such as chlorhexidine, cetyl pyridinium chloride or triclosan have anti-*Candida* activity which is maintained in the presence of nisin. Similarly, nisin has relatively poor activity against *P. gingivalis* whereas tyrothricin is found to have good activity against this bacteria.

Suitably, in compositions of the present invention, nisin is used in a purified form, for instance the product sold under the trade name Ambicin N by Applied Microbiology Inc., 170 53rd Street, Brooklyn, New York, NY 11232, USA.

Suitably, the oral hygiene composition comprises from 0.001 to 5.0%, preferably from 0.005 to 2.0%, advantageously from 0.02 to 1.0 % of nisin, by weight of the composition. In an alternative manner, the level of nisin needed is one which reaches a sufficent level in the oral cavity to inhibit the desired microrganisms. An effective level of nisin, to inhibit the desired organisms, is about 0.99 ppm.

Suitable cationic antimicrobial agents for use in oral hygiene compositions of the invention include:

(i) quaternary ammonium compounds, for instance those in which one or two of the substituents on the quaternary nitrogen has between 8 and 20, preferably between 10 and 18 carbon atoms, and is preferably an alkyl group, which may optionally be interrupted by an amide, ester, oxygen, sulphur, or heterocyclic ring, whilst the remaining substituents have a lower number of carbon atoms, for instance between 1 and 7, and are preferably alkyl, for instance methyl or ethyl, or benzyl, examples of such compounds including benzalkonium chloride, dodecyl trimethyl ammonium chloride, benzyl dimethyl stearyl ammonium chloride, cetyl trimethyl ammonium bromide,

benzethonium chloride (diisobutyl phenoxyethoxyethyl dimethyl benzyl ammonium chloride) and methyl benzethonium chloride;

(ii) pyridinium and isoquinolinium compounds, including hexadecylpyridinium chloride, cetyl pyridinium chloride and alkyl isoquinolinium bromide;

(iii) pyrimidine derivatives such as hexetidine (5-amino-1,3-bis(2-ethylhexyl)-5-methylhexahydropyrimidine);

(iv) amidine derivatives such as hexamidine isethionate (4,4'-diamidino-α, ω-diphenoxyhexane isethionate);

(v) *bis*pyridine derivatives such as octenidine (N,N'[1,10-decanediyldi-1(4H)-pyridinyl-4-ylidene]-*bis*(1-octanamine dihydrochloride); and

(vi) biguanides including:

(a) monobiguanides such as p-chlorobenzyl biguanide, and N'-(4-chlorobenzyl)-N"-(2,4-dichloro-benzyl) biguanide;

(b) *bis*biguanides of the general formula:

$A(X)_zNRC(=NH)NHC(=NH)NH(CH_2)_nNHC(=NH)NHC(=NH)NR'(X')_{z'}A'$

in which:

A and A' which may be the same or different each represent a phenyl group optionally substituted by (C_{1-4})alkyl, (C_{1-4})alkoxy, nitro or halogen, a (C_{1-12})alkyl group, or a (C_{4-12})alicyclic group;

X and X' which may be the same or different each represent (C_{1-3})alkylene;

R and R' which may be the same or different each represent hydrogen, (C_{1-12})alkyl, or aryl(C_{1-6})alkyl;

z and z' which may be the same or different are each 0 or 1;

n is an integer from 2 to 12; and

the polymethylene chain $(CH_2)_n$ may optionally be interrupted by oxygen or sulphur or an aromatic (for instance phenyl or naphthyl) nucleus, and dentally acceptable acid addition salts thereof; in particular chlorhexidine and alexidine and salts thereof such as chlorhexidine digluconate and chlorhexidune acetate; and

(c) poly(biguanides) such as polyhexamethylene biguanide hydrochloride.

Preferred cationic antimicrobial agents include for example, a *bis*biguanide of formula (I), such as chlorhexidine or alexidine, or an orally acceptable acid addition salt thereof, cetyl pyridinium chloride, hexitidine citrate and benzethonium chloride.

Typically, the cationic antimicrobial agent will be present in the range

0.005 to 10%, preferably 0.005 to 5%, more preferably 0.005 to 2.5% by weight of the oral hygiene composition.

Suitable essentially water insoluble non-cationic antimicrobial agents include, for example, halogenated hydroxy diphenyl ethers and thioethers, phenolic and *bis*phenolic compounds, including halogenated salicylanilides, carbanilides, benzoate esters including esters of 2- and 4-hydroxybenzoic acid, and carbanilides, in particular halogenated carbanilides.

Examples of halogenated hydroxy diphenyl ethers include, for example, 3,3'-dibromo-5,5'-dichloro-2,2'-dihydroxydiphenyl ether and 2,4,4'-trichloro-2'-hydroxydiphenyl ether (triclosan); of which triclosan is particularly preferred. Examples of halogenated hydroxy diphenyl thioethers include *bis*(2-hydroxy-3,5-dichlorophenyl)sulphide, and *bis*(2-hydroxy-5-chlorophenyl)sulphide. Examples of phenolic compounds, include, for example, 2-phenylphenol; 4-chlorophenol; 4-chloro-3-methylphenol; 4-chloro-3-methylphenol; 4-chloro-3,5-dimethylphenol; 2,4-dichloro-3,5-dimethylphenol; 5-methyl-2-pentylphenol; 4-isopropyl-3-methylphenol; 5-chloro-2-hydroxydiphenyl-methane; 4',5-dibromosalicylanilide; 3,4',5-tribromosalicylanilide; 2,3,3',5-tetrachlorosalicylanilide; 3,3',4,5'-tetrachloro-salicylanilide; 3,5-dibromo-3'-trifluoromethylsalicylanilide, and 5-n-octanoyl-3'-trifluoromethylsalicylanilide. Examples of *bis*phenolic compounds include, for example, 5,5'-dichloro-2,2'-dihydroxydiphenylmethane; 2,2'-dihydroxy-3,5,6,3',5',5'-hexachlorodiphenylmethane; 2,2'-methylene-*bis*(3,4,6-trichloro-phenol); 2,2'-methylene-*bis*(4-chlorophenol); and 2,2'-methylene-*bis*(4-chloro-6-bromophenol). Examples of benzoate esters include, for example, esters of hydroxybenzoic acid, especially the methyl, ethyl, propyl, isopropyl, butyl, isobutyl, hexyl, heptyl and benzyl esters and phenyl salicylate. Examples of carbanilides include, for example, 3,4,4'-trichlorocarbanilide, 3-trifluoromethyl-4,4'-dichlorocarbanilide, and 3,3',4-trichlorocarbanilide. Preferably, the water insoluble noncationic antimicrobial agent is triclosan.

Typically, the water insoluble noncationic antimicrobial agent will be present in the range 0.005 to 2%, preferably 0.005 to 1%, and more preferably from 0.005 to 0.3% by weight of the composition.

Suitable polypeptide antibiotics (bacteriocins) for use as the antimicrobial agent include gramicidin (Merck Index, 11th edn., entry 4438) and tyrothricin (Merck Index, 11th edn., entry 9745). It will be appreciated that tyrothricin is a polypeptide antibiotic mixture which normally comprises about 10 to 20% gramicidin and from 40 to 60% tyrocidine. Such other

polypeptide antibiotic agent will suitably be present in from 0.001 to 5.0%, preferably from 0.005 to 2.0%, advantageously from 0.02 to 1.0 %, by weight of the composition. In an alternative manner, the level of the polypeptide antibiotic agent needed is one which reaches a sufficent level in the oral cavity to inhibit the desired microrganisms.

Suitable antifungal agents for use in compositions of the present invention include the well known imidazole and triazole agents, such as miconazole, triconazole, or other known agents, such as nystatin. Such antifungal agents will suitably be present in from 0.001 to 1.0%, preferably from 0.01 to 0.5%, more preferably from 0.05 to 0.3% by weight of the composition. Suitable dosage ranges for the antifungal agents are well known in the art for use herein. Miconazole, for instance may be used in an oral dosage form at a level of 125 to 250mg per/dose.

Preferred antimicrobial agents include chlorhexidine and alexidine and salts thereof, in particular chlorhexidine digluconate and chlorhexidine acetate, cetylpyridinium chloride, hexetidine citrate, triclosan, phenyl salicylate, gramidicin and tyrothricin, especially chlorhexidine digluconate, triclosan and cetyl pyridinium chloride.

Antimicrobial agents such as chlorhexidine, cetyl pyridinium chloride and triclosan are active against both bacteria and fungi. In many instances it is found that the concentration of the antimicrobial agent required for antifungal activity is less than that required for antibacterial activity. For instance, at a concentration of about 8.15ppm, cetyl pyridinum chloride kills all the organisms shown in the Example 226, Table 1. In comparison, a level of only about 1.39ppm cetyl pyridinum chloride is needed to inhibit *candida* organisms. Similarly for chlorhexidine and triclosan, the levels needed to inhibit all the desired oral microrganisms are about 8.46ppm and 7.65ppm respectively, whilst the levels for *candida* inhibition are 1.71ppm and about 1ppm, respectively. Thus lower levels of such antimirobial agent may be incorporated into compositions of the present invention if it intended that such antimirobial agent is being included principally to provide anti-Candida activity, to supplement the gap in the spectrum of activity of nisin.

Accordingly, in a further aspect, the present invention provides for an oral hygiene composition comprising an antibacterially effective amount of nisin, preferably in the purified form Ambicin N, and an antimicrobial agent, preferably cetyl pyridinium chloride, chlorhexidine or triclosan, present in an anti-Candida effective amount.

Oral hygiene compositions of the present invention may be presented in any of the formulations conventionally used in the art; for instance, as a mouthwash, dentifrice, including toothpaste and toothpowder, liquid toothpaste, gel, tablet,

lozenge or chewing gum. The components for the orally acceptable carrier or excipient will be selelected according to principles well known to those skilled in the art for the preparation of such formulation types. Such components include a surfactant, thickening agent, humectant and abrasive, as appropriate, as well as other optional extras normally included in an oral care composition. Such components should be compatible with nisin and the antimicrobial agent. Thus, we have previously found (WO 93/11738, SmithKline Beecham) that polypeptide antibiotics such as nisin, gramicidin and tyrothricin are incompatible with anionic surfactants such as sodium lauryl sulphate and sodium N-methyl-N-cocyl laurate which are conventionally used in oral care compositions. Such anionic surfactants should preferably be avoided, in favour of nonionic, cationic or amphoteric surfactants.

Similarly, it is recognised by those skilled in the art that cationic antimicrobial agents such as chlorhexidine and, to a lesser extent, cetyl pyridinium chloride present formulation problems because of their incompatibility, with anionic species normally used in the formulation of oral care compositions, in particular anionic surfactants and, at least for chlorhexidine, anionic thickening agents. These should preferably be avoided. In addition, for dentifrices, care needs to be taken in selecting suitable abrasives, as herein after described.

The term "compatible", when used herein with reference to the selection of a formulation component, is used to indicate that the activity of the antibacterial agent, be it nisin or another antimicrobial agent, is not substantially compromised the presence of the ingredient. Suitably that activity in the presence of the ingredient should not be less than 40%, preferably less than 50%, advantageously less than 60% of that observed in the absence of the ingredient. This may be readily checked by bioassay, for instance a conventional zone diffusion assay against an organism sensitiver to that agent, for instance, *Micrococcus luteus* NCTC 8166 (for triclosan).

Suitable surfactants for use in compositions according to the present invention include, for instance, nonionic, cationic and amphoteric surfactants or mixtures thereof.

Suitable nonionic surfactants include, for example, polyethoxylated sorbitol esters, in particular polyethoxylated sorbitol monoesters, for instance, PEG(40) sorbitan diisostearate, and the products marketed under the trade name 'Tween' by ICI; polycondensates of ethylene oxide and propylene oxide (poloxamers), for instance the products marketed under the trade name 'Pluronic' by BASF-Wyandotte; condensates of propylene glycol; polyethoxylated hydrogenated castor oil, for instance, cremophors: and sorbitan fatty esters.

Suitable amphoteric surfactants include, for example, long chain imidazoline derivatives such as the product marketed under the trade name 'Miranol C2M' by

Miranol; long chain alkyl betaines, such as the product marketed under the tradename 'Empigen BB' by Albright + Wilson, and long chain alkyl amidoalkyl betaines, such as cocamidopropylbetaine, and mixtures thereof.

Suitable cationic surfactants include the D,L-2-pyrrolidone-5-carboxylic acid salt of ethyl-N-cocoyl-L-arginate, marketed under the trade name CAE by Ajinomoto Co. Inc., and cocamidopropyl PG dimonium chloride phosphate and lauramidopropyl PG dimonium chloride phosphate, available under the trade names Monaquat PTC and Monaquat PTL, respectively, from Mona Corporation.

Advantageously, the surfactant is present in the range 0.005 to 20%, preferably 0.1 to 10%, more preferably 0.1 to 5% by weight of the dentifrice.

Suitable thickening agents include, for instance, nonionic thickening agents such as, for example, (C_{1-6})alkylcellulose ethers, for instance methylcellulose; hydroxy(C_{1-6})alkylcellulose ethers, for instance hydroxyethylcellulose and hydroxypropylcellulose; (C_{2-6})alkylene oxide modified (C_{1-6})alkylcellulose ethers, for instance hydroxypropyl methylcellulose; and mixtures thereof. Other thickening agents such as natural and synthetic gums or gum like material such as Irish Moss, gum tragacanth, sodium carboxymethylcellulose, polyvinyl pyrrolidone, starch and thickening silicas are suitable for use in many of the compositions of the present invention, although their use should be avoided in compositions comprising a *bis*biguanide agent such as chlorhexidine or alexidine. Suitably the thickening agent has decreased numbers of anionic groups, such as a carboxy group, although carboxymethyl cellulose may be used. Preferably, the thicking agent is a methylcellulose derivative such as hydroxyethyl cellulose, or hydroxypropyl methylcellulose.

Advantageously the thickening agent is present in the range 0.01 to 30%, preferably 0.1 to 15%, more preferbly 1 to 5%, by weight of the composition.

Suitable humectants for use in compositions of the invention include for instance, glycerine, sorbitol, propylene glycol or polyethylene glycol, or mixtures thereof; which humectant may be present in the range from 5 to 70%, preferably 5 to 30%, more preferably 10 to 30% by weight of the dentifrice. Suitably, when the nonionic thickening agent is hydroxypropyl methylcellulose, the humectant is present in up to 30% by weight of the dentifrice.

Suitable abrasives for use in dentifrice compositions of the present invention include calcium carbonate, calcium phosphates, calcium pyrophosphate, insoluble sodium metaphosphate, sodium aluminosilicate, alumina, hydrated alumina, zinc orthophosphate, plastic particles, and silica, of which silica is the preferred abrasive.

Suitable silicas include natural amorphous silicas, such as, for instance, diatomaceous earth, and synthetic amorphous silicas, such as precipitated silicas and

silica gels, including silica xerogels. Suitable silica xerogels are described in US 3,538,230. Suitable grades of precipitated silicas have BET surface areas in the range 20 to 300, preferably 20 to 100 m^2/g and median agglomerate sizes in the range 2 to 50, preferably 5 to 30μ.

Suitable precipitated silicas and silica xerogels are those marketed under the trade names Sident and Syloblanc, by Degussa and W R Grace Corporation Davison Chemical Division, respectively.

Advantageously, the silica is a "low anion" silica. As used herein, the term "low-anion" silicas refers to those in which anionic impurities such as sodium sulphate and sodium silicate which normally arise during the course of the manufacturing process are kept to a minium, through careful control of the manufacturing process. "Low anion" silicas suitably have less than 1%, preferably less than 0.5% advantageously less than 0.25% by weight of anionic impurities.

Suitable such "low anion" silicas are described in EP 0 368 130 (Proctor & Gamble), EP 0 315 503 and EP 0 396 459 (Rhone-Poulenc) and WO 90/05113 (J.M. Huber Corp). Alternatively, grades of commercially available silica with ionic impurities may be rendered suitable by washing thereof with deionised water. Conductivity measurements on the water after washing may be used to monitor the efficacy of such washing. Suitably the conductivity of the water after washing is reduced to less than 200μSiemens/cm. Suitable "low anion" silicas include the grade RP93 available from Rhone-Poulenc.

Suitably, when the additional antibacterial agent is a *bis*biguanidine derivative, such as chlorhexidine, and the abrasive is a silica derivative, the preferred silica is of the "low anion" type.

Suitably, compositions will have from 5 to 80%, preferably from 10 to 60% by weight of the abrasive.

Suitable dentifrice formulations for compositions comprising chlorhexidine which may be adapted for compositions comprising nisin and chlorhexidine are described in EP 0 364 245-A and EP 0 422 803-A (Beecham Group plc) and EP 0 368 130 (Proctor & Gamble). Suitable dentifrice formulations for compositions comprising cetyl pyridinium chloride which may be adapted for compositions comprising nisin and cetyl pyridinium chloride are described in US 5 176 901 (SmithKline Beecham Corporation).

In a preferred aspect, dentifrice compositions according to the present invention comprise nisin, preferably in the form Ambicin N; an antimicrobial agent selected from cetyl pyridinium chloride, a chlorhexidine salt or triclosan, preferably a chlorhexidine salt or triclosan; a nonionic surfactant such as, for instance, a

polycondensate of ethylene oxide and propylene oxide; a nonionic thickening agent such as, for instance, hydroxypropyl methylcellulose; a humectant such as, for instance, glycerin; and an abrasive such as for instance a "low-anion" silica or a calcium carbonate, optionally in combination with dicalcium *ortho*monophosphate and including an alkaline earth metal metal salt such as calcium chloride.

In a further preferred aspect, a dentifrice composition according to the present invention comprises a nonionic surfactant such as, for instance, a polycondensate of ethylene oxide and propylene oxide, a thickening agent such as sodium carboxymethyl cellulose optionally admixed with a thickening silica, a humectant such as sorbitol optionally admixed with glycerin, and an abrasive such as a "low anion" silica.

Suitable mouthwash formulations will have an aqueous base comprising water or aqueous ethanol, and optionally a further liquid such as glycerin or propylene glycol. A surfactant may also be included, to improve the sensory properties of the composition. Mouthwash compositions may be provided in a "ready to use" form; as a concentrated solution, for dilution by the user immediately prior to use; or in solid form, such as a tablet or in a sachet, for dissolution by the user immediately prior to use. Tablets may suitably be prepared using xylitol and/or sorbitol as the major ingredient. The sachets and tablets may be formulated to provide, on dissolution, a still mouthwash, or, by the incorporation of a suitable effervescent couple, for instance sodium carbonate/bicarbonate and citric acid, an effervescent mouthwash.

Compositions according to the present invention may usefully comprise a fluoride ion source, to provide an anti-caries activity. A fluoride ion source is found to be compatible with nisin. The appropritate fluoride source for the combination of nisin and antimicrobial agent will depend upon the particular antimicrobial agent chosen. The compatabilities, and incompatabilities, are well known and documented in the art for each of the suitable antimicrobial agent. Where applicable, therefore, suitable fluoride ion sources include metal fluoride salts, for instance alkali metal fluoride salts such as sodium fluoride, amine fluoride salts, alkali metal monofluorophosphate salts such as sodium monofluorophosphate and amine monofluorophosphate salts. Suitably the fluoride ion source would, if present, be included to provide from 50 to 3500 ppm, preferably 100 to 2500 ppm of fluoride ions.

In addition to a humectant, compositions of the present invention may also contain further liquid such as, for instance, water, preferably deionised water.

Compositions of the present invention may usefully comprise an orally acceptable chelating agent such as EDTA or citric acid or an alkali metal salt thereof, for instance disodium hydrogen citrate, in accordance with the disclosure of WO

89/12399 (Public Health Research Institute of the City of New York). Suitably, nisin is present in a concentration of from 0.1 to 300μg/ml and the chelating agent present in a concentration of from 0.1 to 20mM.

Nisin comprises the atypical amino acid lanthionine which may be conveniently regarded as two alanine units bonded to a common sulphur atom, to form a thioetther link. This linkage may be vulnerable to proteolysis, leading to deactivation. This is thought to be caused by free radicals which may be generated by certain components of the oral hygiene composition, in particular impurities which may be present in certain components. We have found that some of the grades of nonionic surfactants like the Tweens and some grades of some humectants may cause such a problem. The use of purified grades of formulation ingredients is therefore preferred. In addition, or as an alternative, a competitive substrate, to act as a free radical scavanger, may be usefully included in the composition, for instance, methionine.

The orally acceptable vehicle or carrier may also comprise further optional ingredients such as flavouring agents, sweetening agents, for example sodium saccharin, dyes, whitening agents, for example titanium dioxide, preservatives, antisensitivity agents, such as stronium and potassium salts, and anticalculus agents, such as tetraalkali- and dialkali-imetal pyrophosphate salts. It will be appreciated that in each instance, an optional ingredient, if included, will be compatible with nisin and the antimicrobial agent. It is further appreciated that the combination of additional ingredients such as stronium will be in a manner compatible with other ingredients such as a fluoride ion source.

Compositions according to the invention will have a pH which is orally acceptable and within which the antibacterial activity of nisin is not substantially compromised. Suitably, the pH is in the range 4 to 9.5, preferably in the range 4 to 6.5, more preferably between 4 and 5.5 and most preferably 5 to 5.5.

Compositions according to the invention may be prepared by conventional processes comprising admixing the ingredients together in the appropriate relative amounts in any order that is convenient and finally, and if necessary, adjusting the pH to the desired value.

Compositions of the present invention are intended for use in the prophyllaxis and/or treatment of diseases within the oral cavity. In particular, compositions of the present invention are effective against oral plaque bacterial and as such will be of use in antiplaque therapy. Accordingly, in a further aspect, the present invention also provides a method of reducing or preventing the formation of dental plaque, which method comprises applying an antiplaque effective amount of a composition according to the present invention to a patient in need thereof. Compositions of the

present invention are also useful in the prophyllaxis or treatment of periodontal disease, including gingivitis. Accordingly, the present invention also provides for a method of treating or prophyllaxis of periodontal disease. Certain compositions of the present invention as hereinbefore defined are also of use in treating oral fungal infections. Accordingly, the present invention also provides for a method of treating or prophyllaxis of oral fungal infection.

The invention will now be illustrated by reference to the following examples.

Example 1 - Toothpaste

Ambicin N	0.50%
Triclosan	0.2
Glycerin	22.00
Hydroxypropyl methylcellulose	3.40
Titanium dioxide	1.00
Sodium saccharin	0.25
Poloxamer (Pluronic F108)	2.00
Flavour	1.00
Silica (RP93)	16.00
Deionised water	qs

Further examples can be prepared using 0.1 or 0.3% triclosan.

Example 2 - Toothpaste

Ambicin N	0.50%
Chlorhexidine digluconate	0.5
Glycerin	22.00
Hydroxypropyl methylcellulose	3.40
Titanium dioxide	1.00
Sodium saccharin	0.10
Poloxamer (Pluronic F108)	2.00
Flavour	1.00
Talin	0.02
Silica (RP93)	16.00
Deionised water	qs

Further examples can be prepared using 0.05 or 1.0% chlorhexidine digluconate.

Example 3 - Toothpaste

Ambicin N	0.50%
Cetyl pyridinium chloride	0.5
Glycerin	22.00
Hydroxypropyl methylcellulose	3.40

Titanium dioxide	1.00
Sodium saccharin	0.10
Poloxamer (Pluronic F108)	2.00
Flavour	1.00
Talin	0.02
Silica (RP93)	16.00
Deionised water	qs

Further examples can be prepared using 0.05 % and 1.0 % cetyl pyridinium chloride.

Example 4 - Toothpaste

Ambicin N	0.05%
Triclosan	0.3
Sorbitol (70% soln)	20.00
Glycerin	15.00
Sodium carboxymethyl cellulose	1.20
Sodium fluoride	0.23
Silica (RP 93)	16.00
Thickening silica (Sident 22)	5.00
Sodium saccharin	0.30
Poloxamer (Pluronic F108)	2.00
Deionised water	qs

Further examples can be prepared using 0.1 or 0.2% triclosan.

Example 5 - Toothpaste

Ambicin N	0.05%
Cetyl pyridinium chloride	1.0
Sorbitol (70% soln)	20.00
Glycerin	15.00
Sodium carboxymethyl cellulose	1.20
Sodium fluoride	0.23
Silica (RP 93)	16.00
Thickening silica (Sident 22)	5.00
Sodium saccharin	0.30
Poloxamer (Pluronic F108)	2.00
Deionised water	qs

Further examples can be prepared using 0.05 or 0.5% cetyl pyridinium chloride.

Example 6 - Toothpaste

Ambicin N	0.05%
Triclosan	0.2
Glycerin	22.00

Methocel K15 Premium	0.20
Methocel K100 Premium	3.20
Titanium dioxide	1.00
Sodium saccharin	0.33
Poloxamer (20% Pluronic F108 soln)	10.00
Sodium Fluoride	0.221
Flavour	1.00
Silica (RP 93)	16.00
Deionised water	qs

A further example can be prepared by replacing Methocel K15 and Methocel K100 by sodium carboxymethyl cellulose (1.20%). Additionally, the level of triclosan can be varied using 0.1% or 0.3%.

Example 7 - Toothpaste

Ambicin N	0.05%
Chlorhexidine digluconate	1.0
Glycerin	22.00
Methocel K15 Premium	0.20
Methocel K100 Premium	3.20
Titanium dioxide	1.00
Sodium saccharin (30% soln)	0.33
Poloxamer (20% Pluronic F108 soln)	10.00
Sodium Fluoride	0.22
Flavour	1.00
Talin (5% soln)	0.40
Silica (RP 93)	16.00
Deionised water	qs

A further example can be prepered by replacing the Methocel K15 and Methocel K100 with sodium carboxymethyl cellulose (1.20%). Additionally, the level of chlorhexidine digluconate can be varied using 1.0 % or 0.05 %.

Example 8 - Toothpaste

Ambicin N	0.05%
Cetyl pyridinium chloride	1.0
Glycerin	22.00
Methocel K15 Premium	0.20
Methocel K100 Premium	3.20
Titanium dioxide	1.00
Sodium saccharin (30% soln)	0.33
Poloxamer (20% Pluronic F108 soln)	10.00

Sodium Fluoride	0.221
Flavour	1.00
Talin (5% soln)	0.40
Silica (RP 93)	16.00
Deionised water	qs

A further example can be prepared by replacing the Methocel K15 and Methocel K100 with sodium carboxymethyl cellulose (1.20%). Additionally, the level of cetyl pyridinium chloride can be varied using 0.05 % or 0.5 %.

Example 9 - Mouthwash

Ambicin N	0.030%
Triclosan	0.020
Glycerin	5.000
Flavour	0.075
Ethanol (96%)	15.00
Soluble saccharin	0.010
Sodium Fluoride	0.023
Colouring	0.100
Propylene Glycol	15.00
Deionised water	qs

Further examples can be prepared using triclosan at 0.01% or 0.03%.

Example 10 - Mouthwash

Ambicin N	0.030 %
Triclosan	0.03
Glycerin	30.000
Flavour	0.075
Ethanol (96%)	15.00
Cremophor RH60	0.60
Soluble saccharin	0.005
Sodium Fluoride	0.023
Colouring	0.10
Deionised water	qs

In an alternative formulation, Cremophor RH60 may be replaced by propylene glycol. Further, the level of triclosan may also be varied using 0.005, 0.01% or 0.02%.

Example 11 - Mouthwash

Ambicin N	0.030%
Chlorhexidine digluconate	0.01
Glycerin	5.000
Flavour	0.075

Ethanol (96 %)	5.00
Soluble saccharin	0.010
Sodium Fluoride	0.023
Colouring	0.100
Cremophor RH60	0.1
Deionised water	qs

A further example may be prepared using Cremophor RH60 at 0.2%. In addition, the level of chlorhexidine digluconate may be varied, using 0.05%, 0.1% or 0.2 %.

Example 12 - Mouthwash

Ambicin N	0.030%
Cetyl pyridinium chloride	0.01%
Glycerin	5.000
Flavour	0.075
Ethanol (96%)	5.00
Soluble saccharin	0.0001
Sodium Fluoride	0.023
Colouring	0.001
Cremophor RH60	0.1
EDTA	0.004
Deionised water	qs

A further examples may be prepared using Cremophor RH60 at 0.2%. In addition, Cremophor RH60 may be omitted or replaced by a nonionic surfactant such as Tween 20 or TritonX100 (0.2%). Other examples can be prepared using 0.05, 0.1 or 0.2 % cetyl pyridinium chloride.

Example 13 -Mouthwash

Ambicin N	0.030%
Triclosan	0.005
Disodium hydrogen citrate	0.263
Ethanol (96%)	15.00
Propylene glycol	15.00
Glycerin	30.00
Deionised water	qs

Further examples may be prepared in which the level of triclosan is 0.01%, 0.02%, or 0.03%.

Example 14 - Mouthwash

Ambicin N	0.030%
Chlorhexidine digluconate	0.005
Disodium hydrogen citrate	0.263

Deionised water	qs

Further examples may be prepared using chlorhexidine digluconate at 0.05%, 0.1% or 0.2% levels.

Example 15 - Mouthwash

Ambicin N	0.030%
Cetyl pyridinium chloride	0.01
Disodium hydrogen citrate	0.263
Deionised water	qs

Further examples may be prepared using 0.05, 0.1 or 0.2% of cetyl pyridinium chloride.

Example 16 - Mouthwash (concentrated formulation)

Ambicin N	1.0%
Phenyl salicylate	1.0
Propylene glycol	73.00
Ethanol (96%)	15.00
Flavour	2.91
Sodium Saccharin	0.11
Tween 20	0.5
Deionised water	6.48

The concentrated mouthwash is used by diluting a few drops (about 1ml) into a half-filled glass of water (approx. 100ml) which is gargled by the user.

Example 17 - Mouthwash (concentrated formulation)

Using the formulation of example 16 but replacing phenyl salicylate with cetyl pyridinium chloride at between 0.075 to 1.5%, for instance 0.75%.

Example 18 - Mouthwash (concentrated formulation)

Using the formulation of example 16 but replacing phenyl salicylate with chlorhexidine digluconate at between 0.075 to 1.5%, for instance 0.75%.

Example 19 - Mouthwash tablet

Ambicin N	0.9%
Triclosan	0.9
Sodium Fluoride	1.5
Magnesium Stearate	1.0
Flavour (spray dried)	1.5
Colouring	0.05
Citric Acid (anhydrous)	15
Sodium Bicarbonate	11
Sodium Carbonate	10
Xylitol	qs

Further examples may be prepared using replacing xylitol with sorbitol or a mixture of xylitol and sorbitol. The ingredients are admixed and compressed into 500mg tablets. The citric acid, sodium bicarbonate and sodium carbonate are preferably admixed with the xylitol or sorbitol first. Further examples may be prepared using 0.1% triclosan. In use, the tablet is disolved in about 100ml of water, to form an effervescent mouthwash solution which is then gargled by the user.

Example 20 - Mouthwash tablet

Using the formulation of example 19 but replacing triclosan with chlorhexidine acetate at 0.9 or 0.075 %.

Example 21 - Mouthwash tablet

Using the formulation of example 19 but replacing triclosan by cetyl pyridinium chloride at 1.5 or 0.075 %.

Example 22 - Sachet - Effervescent Mouthwash

Ambicin N	0.225%
Triclosan	0.225
Sodium Fluoride	.375
Menthol	0.5
Citric Acid (anhydrous)	15.0
Sodium Bicarbonate	11.0
Sodium Carbonate	10.0
Xylitol	qs to 100

Xylitol may be replaced by sorbitol or a sorbitol/xylitol mixture. The ingredients are admixed and placed into 2 g dosage packets. The sodium bicarbonate and sodium carbonate are preferably admixed separately with xylitol or sorbitol first. The level of the effervescent couple may be increased to citric acid (22.5%), sodium bicarbonate (16.5%), and sodium carbonate (15%). Further examples may be prepared in which the level of triclosan is 0.0225%.

Example 23 - Sachet - Effervescent Mouthwash

Using the formulations of example 22 but replacing triclosan by cetyl pyridinium chloride at from 0.05 to 0.375 %.

Example 24 - Sachet - Effervescent Mouthwash

Using the formulations of example 22 but replacing triclosan with chlorhexidine acetate at from 0.025 to 0.225%.

Example 25 - Antibacterial spectrum of Ambicin N

The antibacterial spectrum of activity of Ambicin N (Amb) was determined by testing the compound against a range of orally important Gram negative and Gram positive bacteria in a conventional nominal inhibitory concentration (NIC) assay and compared with that of cetyl pyridinium chloride (CPC), chlorhexidine (CHX) and

triclosan. The results are reported in Table 1. In most instances, Ambicin N has superior activity but as can also be noted Ambicin N lacks effective inhibitory activity against *candida* species. In comparison, CPC, CHX and TCN are all effective against *Candida*.

Table 1 - NIC data

Organism	CPC (ppm)	CHX (ppm)	TCN (ppm)	Amb (ppm)
Strep. agalactiæ	0.78	0.41	4.96	0.22
Strep. sanguis	2.12	0.86	0.88	0.23
Strep.mutans	3.10	3.94	1.11	0.43
Strep. milleri	4.58	1.58	1.08	0.14
Strep. mitis	2.23	1.50	3.94	0.33
Strep mitior	1.22	4.55	4.23	0.05
Strep. salivarius	2.36	1.44	4.05	0.18
Strep.pyogenes	0.78	0.16	4.35	0.009
Staph.aureus	0.66	0.54	0.09	0.13
G.vaginalis	0.3	1.3	0.41	0.22
Lacto odontolyticus	0.83	1.88	7.49	0.02
Act. odontolyticus	1.23	3.37	3.02	0.12
Act. israelii	8.15	4.11	3.19	0.53
Act. naeslundii	4.61	2.01	1.98	>12.8
Act. actinomycetem	1.02	0.53	1.69	0.02
Fuso. nucleatum	0.82	0.64	1.6	0.048
Bact. intermedius	4.68	2.49	3.72	0.91
Peptostrepto. micros	4.96	8.46	6.76	>1.28
Porph. gingivalis	0.94	2.36	4.00	>1.28
Bact. ureolyticus	0.56	0.69	7.65	0.035
Candida albicans	1.39	1.71	1.00	>128
Candida kefyr	0.61	0.42	0.46	>128
Candida tropicalis	0.48	0.98	0.35	>128

Example 26 - Antibacterial spectrum of Ambicin N

The antibacterial activity of a solution comprising Ambicin N (Amb) (500ppm) in the presence or absence of one of cetyl pyridinium chloride (CPC), chlorhexidine (CHX) or triclosan (TCN) (500ppm) was assayed in a conventional zone difusion assay against the important oral microorganisms *S sanguis*, *S mutans*, *A actionmyces*, *W*

recta, *F nucleatum*, *C albicans*, *C kefyr* and *C tropicalis*. The assay was repeated for each of cetyl pyridinium chloride, chlorhexidine or triclosan individually. The results are presented in tables 2 and 3. Although there is no evidence to suggest any degree of synergy between Ambicin N and any of cetyl pyridinium chloride, chlorhexidine or triclosan, the data also shows that there is no reduction in the activity of either agent in the presence of the other; ie, there is mutual compatibility.

Table 2: Zone Diffusion Assay - mean zones obatained against various oral bacteria (mm)

	S sanguis NCTC 10904	S mutans NCTC 11061	A actino-myces NCTC 9709	W recta NCTC 11489	F nucleatum NCTC 10562
Ambicin N	15.38	18.36	23.04	28.19	29.38
TCN	28.97	32.82	35.92	37.20	33.98
CPC	14.44	17.05	19.41	17.15	18.99
CHX	22.76	29.68	35.68	32.35	36.97
Amb/TCN	26.73	31.47	31.28	33.08	26.11
Amb/CPC	21.92	29.42	35.25	31.52	37.33
Amb/CHX	14.48	17.05	19.57	20.48	19.25

Table 3: Zone Diffusion Assay - mean zones obatained against various *Candida* sp (mm)

	C kefyr NCTC 3106	C tropicalis NCTC 3114	C albicans NCTC 3089
Ambicin N (Amb)	0	0	0
TCN	21.99	13.66	16.99
CPC	10.43	10.39	9.79
CHX	17.03	17.36	14.93
Amb/TCN	18.66	11.77	16.74
Amb/CPC	16.21	17.45	14.58
Amb/CHX	10.66	9.98	9.74

Claims

1. An oral hygiene composition which comprises an antibacterially effective amount of nisin, an antimicrobially effective amount of an antimicrobial agent and an orally acceptable carrier or excipient.

2. A composition as claimed in claim 1 in which nisin is used in a purified form.

3. A composition as claimed in claim 2 in which the purified form of nisin is Ambicin N.

4. A composition as claimed in any one of claims 1 to 3 in which the antimicrobial agent is a cationic antimicrobial agent, a non-cationic antimicrobial agent, another (non-nisin) polypeptide antibiotic (bacteriocin) or an antifungal agent.

5. A composition as claimed in any one of claims 1 to 4 in which the cationic antimicrobial agent is chlorhexidine, or an orally acceptable acid addition salt thereof, or cetyl pyridinium chloride.

6. A composition as claimed in any one of claims 1 to 4 in which the noncationic antimicrobial agent is triclosan.

7. A composition as claimed in claim 5 or 6 in which the antimicrobial agent is present in an anti-Candida effective amount.

8. A composition as claimed in any one of claims 1 to 4 in which the polypeptide antibiotic is gramicidin or tyrothricin.

9. A composition as claimed in any one of claims 1 to 8 in which the orally acceptable carrier or excipient comprises a surfactant which is a nonionic, cationic or amphoteric surfactant or a mixture thereof.

10. A composition as claimed in any one of claims 1 to 9 in which the orally acceptable carrier or excipient comprises a nonionic thickening agent or a natural or synthetic gum or gum-like material.

11. A composition as claimed in any one of claims 1 to 10 which is a dentifrice and in which the abrasive is a silica abrasive.

12. A composition as claimed in claim 11 in which the silica abrasive is a low anion silica, comprising less than 1% by weight of the abrasive of anionic impurities.

13. A composition as claimed in any one of claims 1 to 10 which is a dentifrice which comprises nisin; an antimicrobial agent selected from cetyl pyridinium chloride, a chlorhexidine salt or triclosan; a nonionic surfactant; a nonionic thickening

agent; a humectant; and an abrasive which is a "low-anion" silica or calcium carbonate optionally in combination with dicalcium *ortho*monophosphate and including an alkaline earth metal metal salt such as calcium chloride.

14. A composition as claimed in any one of claims 1 to 10 which is a dentifrice which comprises nisin; an antimicrobial agent selected from cetyl pyridinium chloride or triclosan; a nonionic surfactant; a thickening agent which is sodium carboxymethyl cellulose optionally admixed with a thickening silica; a humectant; and an abrasive which is a "low anion" silica.

15. A composition as claimed in any one of claims 1 to 10 which is a mouthwash.

16. A composition as claimed in any one of the preceding claims which further comprises an orally acceptable chelating agent.

17. A composition as claimed in claim 16 in which the orally acceptable chelating agent is EDTA, citric acid or an alkali metal salt thereof.

18. A composition as claimed in any of the preceding claims which further comprises methiononine.

19. A process for preparing an oral hygiene composition as defined in any of the preceding claims which process comprises admixing the ingredients in apprpriate quantities, any order that is convenient, and thereafter, and if necessary, adjusting the pH to give the desired final value.

20. A composition as defined in any one of claims 1 to 18 for use in oral therapy.

21. The use of nisin, an antimirobial agent and an orally acceptable excipient or carrier in the manufacture of an oral care composition for treating or the prophyllaxis of plaque and/or periodontal disease.

22. The use of nisin, an antimirobial agent selected from chlorhexidine or a salt thereof, cetyl pyridinium chloride or triclosan and an orally acceptable excipient or carrier in the manufacture of an oral care composition for treating or the prophyllaxis of oral fungal infection.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 93/02387

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K7/16 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,90 09739 (THE PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC.) 7 September 1990 see the whole document ---	1-4,9, 10, 15-17, 19-21
X,P	EP,A,0 545 911 (APPLIED MICROBIOLOGY, INC.) 9 June 1993 see the whole document & WO,A,89 12399 (PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC.) cited in the application ---	1-4,9, 15-17, 19-21
A	DE,A,27 55 052 (MED-CHEM LABORATORIES) 29 June 1978 see the whole document ---	1-4
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 March 1994	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Fischer, J.P.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 93/02387

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA,A,2 058 455 (HAARMANN & REIMER CORP.) 22 June 1992 see the whole document	1-4,15, 19-21
A	CA,A,2 055 984 (HAARMANN & REIMER CORP.) 22 June 1992 see page 2, line 19 - line 29; claims 1,3,4	1-4,15, 19-21
A	GB,A,2 160 771 (LION CORPORATION) 2 January 1986 see example 8	1-4, 9-12,15, 19-21
A	EP,A,0 140 498 (LION CORPORATION) 8 May 1985 see the whole document	1-4, 9-12,15, 19-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten. ..al Application No
PCT/GB 93/02387

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9009739	07-09-90	US-A-	4980163	25-12-90
		AU-B-	618714	02-01-92
		AU-A-	5285090	26-09-90
		CA-A-	2028140	02-09-90
		EP-A-	0424484	02-05-91
		JP-T-	3504864	24-10-91
EP-A-0545911	09-06-93	AU-B-	631803	10-12-92
		AU-A-	3843089	12-01-90
		EP-A-	0382814	22-08-90
		JP-T-	3500051	10-01-91
		WO-A-	8912399	28-12-89
		US-A-	5135910	04-08-92
		US-A-	5217950	08-06-93
		US-A-	5260271	09-11-93
WO-A-8912399	28-12-89	AU-B-	631803	10-12-92
		AU-A-	3843089	12-01-90
		EP-A-	0382814	22-08-90
		EP-A-	0545911	09-06-93
		JP-T-	3500051	10-01-91
		US-A-	5135910	04-08-92
		US-A-	5217950	08-06-93
		US-A-	5260271	09-11-93
DE-A-2755052	29-06-78	AU-A-	3125677	14-06-79
		JP-A-	53091126	10-08-78
CA-A-2058455	22-06-92	JP-A-	4334307	20-11-92
CA-A-2055984	22-06-92	JP-A-	4295431	20-10-92
GB-A-2160771	02-01-86	JP-A-	61012629	21-01-86
		CA-A-	1275380	23-10-90
		DE-A,C	3522898	02-01-86
		US-A-	4714612	22-12-87
EP-A-0140498	08-05-85	JP-C-	1730543	29-01-93
		JP-B-	4021649	13-04-92
		JP-A-	60038329	27-02-85

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. ...al Application No
PCT/GB 93/02387

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0140498		US-A- 4693888	15-09-87